

Genotyping Protocol: MMRRC line 352

Assay Type: PCR - can not distinguish heterozygous animals from homozygous animals

DNA Extraction: DNA from tail snips was extracted using Qiagen's DNeasy kit. Kit directions for animal tissues were performed with a few minor modifications as follows: repeat AW1 and AW2 wash steps one time, elute in 200µl of AE buffer once.

Primer Information:

- 1) Name: m352 F Sequence: AAC TAG TGC TTG CCC TGC TGA GAT
2) Name: m352 WT R Sequence: TCA GAG ATT CAC CTG CCA CTG GTT
2) Name: m352 KO R Sequence: CGC GAG CTC AAT TAA CCC TCA CTA

Primer location: m352 F & WT R: Mus musculus LCAD gene intron

Primer location: m352 KO R: Insertion plasmid sequence

PCR Master Mix Components:

component	manufacturer	concentration	µl/rxn
buffer	Roche	10X	2
dNTP	Roche	1.25mM	3.2
M352 F	IDT	25µM	0.3
M352 WT R	IDT	25µM	0.3
M352 KO R	IDT	25µM	0.3
FastStart taq	Roche	5 U/µl	0.2
sterile water			12.7

PCR Setup:

Final Reaction: 19µl master mix & 1µl DNA template

All reactions were performed in 200µl thin walled PCR tubes and were run in Perkin Elmer 2400 thermocycler or Applied Biosystems 2700 thermocycler.

Cycle Parameters:

- 1) 94°C 5 minutes
- 2) 94°C 30 seconds
- 3) 66°C 30 seconds
- 4) 72°C 30 seconds
- 5) Repeat steps 2-4 34 times for a total of 35 cycles
- 6) 72°C 7minutes
- 7) 4°C hold until refrigerate product

Product Analysis:

All products were analyzed on a 3% agarose gel with ethidium bromide staining

Knockout product (both homozygous mutant and heterozygous mutant) = 292 bp & 114 bp

WT product = 292 bp